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Epidermal growth factor receptor inhibition in lung cancer: the evolving role of individualized therapy

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Abstract

Non-small cell lung cancer (NSCLC) is the major cause of cancer-related deaths in the USA and worldwide. Most patients present with advanced disease, and treatment options for these patients are generally limited to platinum-based chemotherapy and a few targeted therapies. Targeted agents currently in use for NSCLC inhibit oncogenic receptor tyrosine kinase pathways, such as the epidermal growth factor receptor (EGFR) pathway. While current EGFR-targeted agents, including erlotinib and gefitinib, may result in dramatic responses, they demonstrate efficacy in only a fraction of patients, and resistance to these agents frequently develops. In order to select patients most likely to benefit from blockade of EGFR pathways, investigators have focused on identifying molecular correlates of response to anti-EGFR therapy. New strategies to minimize the risk of resistance to EGFR inhibition have been employed in the development of next-generation EGFR tyrosine kinase inhibitors, such as PF00299804 and BIBW 2992; these include irreversibility of target binding, inhibition of multiple EGFR family receptors, and/or simultaneous inhibition of EGFR and other oncogenic pathways.

Keywords

Epidermal growth factor receptor; NSCLC; Targeted therapy; Resistance

1 Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide, accounting for more than 1 million deaths each year [1]. In the USA, lung cancer accounts for approximately 28% of all cancer-related deaths [2]. Non-small cell lung cancer (NSCLC) is the most common type and accounts for at least 85% of all lung cancer cases [2, 3]. Treatment options for NSCLC depend on stage of disease and include surgery, radiation, platinum-based doublet chemotherapy, and targeted therapies in some cases [3]. Most patients present with advanced or metastatic disease, for which chemotherapy is generally recommended as first-line treatment [3]; however, efficacy is modest and therapy is associated with significant toxicity [4, 5]. Adding bevacizumab, an anti-angiogenic agent, to standard first-line doublet chemotherapy regimens has been shown to increase efficacy, but with only minimal improvements in clinical outcomes [6, 7].

Epidermal growth factor receptor (EGFR) inhibitors have been investigated as first-line or subsequent therapy options for patients with NSCLC. Recent efforts are focused on

identifying specific molecular markers that may predict treatment response, thus allowing for a more tailored approach for treating patients with NSCLC. This article will provide an overview of current understanding of the implications of EGFR signaling in the treatment of NSCLC and highlight strategies for its inhibition, with a focus on EGFR tyrosine kinase inhibitors (TKIs).

2 The EGFR signaling pathway

The EGFR family of receptor tyrosine kinases, also known as the HER or ErbB family, has four known members: EGFR or HER1/ErbB1, HER2/ErbB2, HER3/ErbB3, and HER4/ErbB4 [8]. Downstream signaling regulated by this family of receptor tyrosine kinases (RTKs) is complex and multidimensional, and aberrant activation of the pathway leads to downstream events that stimulate five of the six hallmarks of cancer, including evasion of apoptosis, self-sufficient growth, insensitivity to anti-growth signals, sustained angiogenesis, and tissue invasion and metastasis (the remaining hallmark is limitless replicative potential, which is not promoted by *EGFR* activation) [9] (Fig. 1). EGFR is overexpressed in many epithelial cancers, including NSCLC [10]; small cell lung cancer is one of the few solid tumors in which EGFR is not overexpressed [11]. Thus, EGFR has been the most intensively studied of the four family members, and has become a prototype of classical RTKs. However, deregulation of the pathway may occur at several nodal points, providing a multitude of targets for selection of individualized therapy [12]. Investigation of signaling pathways downstream of EGFR has demonstrated the far-reaching effect of this pathway on diverse cellular processes, such as proliferation, angiogenesis, and development [8].

Regulation of the EGFR pathway is complex, and a comprehensive review is beyond the scope of this article. Like other HER family members, EGFR is a transmembrane receptor activated in response to ligand (EGF and others) binding to the extracellular domain [13]. Ligand binding induces conformational changes that allow for the formation of receptor dimers. Both homodimer and heterodimer formation within the EGFR family have been verified, and the variety of pairing combinations is thought to provide an additional layer of signaling regulation. Activation of the kinase domain of the receptor leads to autophosphorylation and activation and the subsequent recruitment of adaptor proteins that mediate downstream signaling [13]. The EGFR pathway is also regulated on a higher level by several feedback loops. For instance, activation leads to increased cellular production of ligand and increased receptor internalization [13].

EGFR activates two major downstream intracellular signaling pathways—the Ras-Raf-mitogen-activated protein kinase (MEK)-mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K)-Akt/protein kinase B-mammalian target of rapamycin (mTOR) cascades [14–16]. The Ras-Raf-MEK-MAPK pathway modulates several cellular processes including gene transcription, G1/S cell-cycle progression, and cellular proliferation. EGFR tyrosine kinase activity leads to activation of the small GTPase Ras, which then exchanges GDP for GTP; activated, GTP-bound Ras then stimulates the Raf-MEK-MAPK cascade [17]. The PI3K pathway regulates anti-apoptotic and prosurvival signal cascades [17]. These pathways may also be modulated by other proteins such as c-mesenchymal-epithelial transition factor (MET), insulin-like growth factor 1 receptor (IGF-1R), LKB1-amp-activated protein kinase [14], and the echinoderm microtubule-associated protein-like 4/anaplastic lymphoma kinase (EML4-ALK) fusion protein [18]. While EML4-ALK is detected in less than 10% of lung cancers, it is most common in adenocarcinomas and in never or light smokers [14, 18]. It is also almost never detected along with activating *EGFR* or V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations [18], suggesting that EML4-ALK is involved in this pathway. As a

result, ALK fusion proteins are being investigated as potential therapeutic targets for NSCLC treatment [18].

Other downstream targets of EGFR include the phospholipase C-protein kinase C (PKC) and Janus kinase/signal transducers and activators of transcription (STAT) pathways [16]. Phospholipase C enzymatically cleaves phosphatidyl inositol 4,5-bisphosphate, which leads to release of cellular calcium stores and activation of PKC [19]. PKC in turn activates the Raf-MEK-MAPK pathway and other effector proteins [17]. STATs, which stimulate transcription of nuclear factors that promote cell survival and oncogenesis [17], are activated by EGFR signaling both directly by interaction with EGFR [20] and indirectly through Src family kinases [21]. Many protein families (e.g., Ras, Raf) contain multiple members, adding to the complexity and scope of activation of these pathways through EGFR. As known cellular processes influenced by EGFR signaling have continued to grow, the effects of the complete biochemical network associated with EGFR are not entirely known.

3 Implications of EGFR expression and activity in NSCLC

Owing to the variety of cellular processes regulated by EGFR signaling, its deregulation has been associated with carcinogenesis [22]. Aberrant activation of the EGFR pathway is thought to be due to at least three mechanisms: (1) enhanced production of ligands by cancer cells, (2) increased expression of EGFR on the cancer cell membrane, and (3) activating mutations of the *EGFR* gene or other family members [12, 15]. Several strategies for inhibition of EGFR, including tyrosine kinase inhibition, have been developed for treatment of human cancers, including lung cancer. EGFR is frequently overexpressed in NSCLC, and EGFR overexpression has been associated with poor prognosis [23, 24]. Total EGFR protein is detectable in approximately 80–85% of patients with NSCLC, though levels of expression vary widely on a continual scale [3]. Efforts toward implementing routine molecular profiling of tumors have been underway, with the hope that relevant correlates may predict patient response to EGFR blockade.

EGFR protein levels can be measured using several methods, including radioactive-labeled ligand binding, competitive immunoassay, western blotting, and immunohistochemistry (IHC) [25]. With the exception of IHC, however, these methods require complex laboratory equipment and are not easily modified for clinical use [25]. Moreover, analysis of EGFR levels by IHC is affected by many variables that decrease its reproducibility and quantitative value [26], and its use has been inconsistent in predicting response to EGFR TKIs [27–30]. Currently, IHC is not yet optimized for determining patient eligibility to receive EGFR TKI therapy, though standardization of this methodology may allow for clinical use in the future. As the phosphorylated form of the protein is the active form, measurements of phospho-EGFR may provide more clinically relevant information; however, the half-life of the phosphorylated form is short, and unless a specimen is optimally collected and processed, phospho-EGFR measurements may result in misleading findings [31].

Increased expression may be due to increased gene copy number via amplification or polysomy. Thus, increased copy number of *EGFR* in tumors may be meaningful for predicting response to EGFR TKIs. Although polysomy and amplification are often regarded as being of similar importance, chromosome 7 is the site not only of the *EGFR* gene but also of *BRAF* and *MET*. Therefore, polysomy may activate genes that have opposite effects on the EGFR pathway, resulting in complex interactions. Several methods are available to measure *EGFR* gene copy number, including fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH), and real-time polymerase chain reaction (PCR). FISH is widely used to detect copy number of specific genes in tissue sections [32], and *EGFR* gene amplification detected using this method has been shown to

correlate with response to EGFR-targeted agents [27, 33, 34]. Gene number determined by FISH appears to be a more robust predictive marker of EGFR-TKI response than EGFR protein expression measured by IHC, but more data are needed regarding reproducibility and predictive power across laboratories [26]. In addition, FISH (and CISH) can distinguish between amplification and polysomy. There is also the potential that combined readouts from IHC and FISH may predict response to EGFR TKIs [35]. CISH, an alternative to FISH for measuring *EGFR* copy number, appears to be an accurate and reproducible method [26], but requires further evaluation and validation in clinical trial samples [25]. Finally, real-time PCR is a potential tool for detecting *EGFR* copy number [26]; however, measurements do not necessarily correlate with FISH, IHC, or mRNA expression [36]. Another method to detect increased copy number, in particular amplification, is comparative genomic hybridization (CGH), although it is not suitable for routine clinical use. An *in vitro* study using NSCLC cell lines demonstrated that FISH, quantitative polymerase chain reaction, and CGH gave comparable results [37]. Additionally, studies to date have not consistently shown that *EGFR* copy number is predictive of response to treatment, disease control, or progression-free or overall survival [36, 38–40].

There is growing evidence that *EGFR* mutations may be predictive of therapeutic efficacy [3]. The evidence that mutations predict for progression-free survival is much greater than for overall survival [12]. These findings may, in part, be related to the fact that TKI therapy is often a second- or third-line option. The most common *EGFR* mutations are deletions in exon 19 or a single point mutation in exon 21, both of which cause activation of the tyrosine kinase domain [41]. Presence of these activating mutations is predictive of treatment benefit from EGFR TKI therapy [26, 42–52]; however, the *de novo* existence or acquisition of some *EGFR* mutations (e.g., T790M) are associated with EGFR TKI resistance [53]. There are many techniques that may potentially be used for *EGFR* mutation analysis, the majority of which are PCR based [25, 26]. Mutations can be detected using a PCR assay and then confirmed by DNA sequencing [54]. Some large clinical screens for *EGFR* mutations have been performed [52]; however, more streamlined approaches are in development. For instance, it was recently demonstrated that detection of shed tumor DNA using the DxS *EGFR* mutation test kit [55] from the plasma of patients is sufficient for determination of *EGFR* mutation status; *EGFR* mutation status was also associated with patient outcome in this study [56]. Other potential indicators, such as *KRAS* mutations, *EGFR* truncations, expression levels of MET and HER2, and Akt phosphorylation state are also being investigated as predictors of response to EGFR-directed therapy [57].

Though various methodologies are available to assess potential molecular markers predictive of response to anti-EGFR therapy [26], more advancements will be necessary before these may provide widespread benefit to patients. As agents are developed that target downstream mediators of EGFR signaling, other mutational and expression assays will likely be evaluated. Ongoing randomized studies will continue to validate the assays that can predict patient outcome. It is likely that as molecular characteristics more routinely dictate treatment decisions, pathologists will begin playing a larger role in choosing the optimal therapy for individual patients [58]; testing of new biopsies when NSCLC patients relapse or begin a new treatment regimen will also be of importance. It is hoped that in the near future, extensive testing of patient tumors will become the standard of care for making treatment decisions. The ability to identify appropriate biomarkers to predict clinical efficacy would render clinicians one step closer to the provision of personalized medicine for patients with NSCLC.

4 Drugs targeting the EGFR pathway

Two classes of EGFR inhibitors, monoclonal antibodies (e.g., cetuximab) and small-molecule TKIs (e.g., erlotinib, gefitinib), have been studied in phase III trials and are currently in clinical use in NSCLC [15, 59–63]. Monoclonal antibodies targeting EGFR bind to the extracellular domain of EGFR and block ligand binding and receptor activation, while small-molecule EGFR TKIs compete reversibly with ATP to bind to the catalytic domain of the intracellular kinase domain to inhibit its activity.

Results from the phase III FLEX trial ($N=1,125$) showed that cetuximab in combination with chemotherapy (vinorelbine/cisplatin) improved overall survival compared with chemotherapy alone (11.3 vs. 10.1 months; $P=0.04$) in patients (performance status 0–2) with previously untreated advanced NSCLC that expressed EGFR [63]. Cetuximab is not approved by the US Food and Drug Administration (FDA) for NSCLC, but is recommended by the National Comprehensive Cancer Network (NCCN) in combination with vinorelbine/cisplatin for patients with advanced disease whose tumors express EGFR [3]. The Committee for Medicinal Products for Human Use, the scientific committee of the European Medicines Agency, has adopted a negative opinion for the use of cetuximab for such patients [64].

Early clinical data showed that approximately 10% of unselected patients with NSCLC respond to gefitinib or erlotinib, possibly reflective of the fact that 10–15% of patients have activating *EGFR* mutations [30]. In a phase III study of patients ($N=1,692$) with refractory, advanced NSCLC [65], gefitinib did not show an overall survival benefit compared with placebo. As a result, gefitinib currently has a limited indication in the USA for the continued treatment of patients benefiting from gefitinib therapy. Efforts to determine the response to gefitinib in selected patient populations are ongoing. In contrast, erlotinib has shown significantly longer progression-free survival (2.2 vs. 1.8 months; hazard ratio [HR], 0.61; $P<0.001$) and overall survival (6.7 vs. 4.7 months; HR, 0.70; $P<0.001$) compared with placebo in patients with advanced NSCLC who had received prior chemotherapy [66]. Erlotinib is indicated by the US FDA for treatment of locally advanced or metastatic NSCLC that has progressed after at least one line of chemotherapy [67]. Erlotinib is recommended by the NCCN as second- and third-line therapy for NSCLC; it is also recommended for first-line treatment in patients with *EGFR* mutations, but this is supported by a low level of evidence [3]. The potential for primary resistance to currently available EGFR TKIs is an important consideration for treatment, but even in patients who initially respond, relapse often eventually occurs.

4.1 Major mechanisms of acquired resistance to EGFR TKIs

Resistance is considered primary when patients are initially refractory to treatment and acquired when patients experience an initial but not lasting response to treatment. Activating *EGFR* mutations may predict treatment benefit from EGFR TKIs, while other secondary *EGFR* mutations have been associated with acquired resistance [68–70]. *KRAS* mutation has also been associated with a poor response to EGFR TKIs [71, 72].

Two mechanisms of delayed resistance to EGFR TKIs may be associated with changes in EGFR itself or with changes in other proteins (Fig. 2). The T790M mutation has been reported in 50% of EGFR TKI-resistant tumors [69, 73] (reviewed in [74]). Recent data suggest that T790M can be detected before TKI treatment using highly sensitive assays [56, 75], supporting the concept that treatment pressure selects for mutations that are actually present *de novo*, but perhaps in a very small percentage of tumor cells. Other secondary *EGFR* point mutations associated with development of resistance, such as D761Y, have also been reported [73].

Bypass of EGFR signaling can also occur through changes in other proteins, which contribute to resistance in some NSCLC patients. Amplification of the proto-oncogene *MET*, an RTK that shares some downstream effectors with *EGFR*, has been observed in approximately 20% of patients resistant to EGFR TKIs [76]. Since the proportion of resistant tumors having *EGFR* mutations (50%) and *MET* amplification (20%) do not account for all patients, other mechanisms of resistance may exist. Considering the complexity of these signaling networks, increased signaling through other HER family members or through overlapping pathways such as the IGF-1R, fibroblast growth factor receptor, vascular endothelial growth factor receptors (VEGFRs), and platelet-derived growth factor receptors is possible [77–83]. To address the problem of resistance to gefitinib and erlotinib, agents that bind targets irreversibly, inhibit multiple members of the same receptor family, and inhibit members of multiple receptor families to target more than one process (e.g., proliferation and angiogenesis), are currently in development for the treatment of NSCLC.

5 Drugs of the future: targeting multiple points in the EGFR pathway

Many strategies to inhibit EGFR signaling exist in addition to direct targeting of the receptor. Points within the pathway that are currently being investigated for intervention in NSCLC are illustrated in Fig. 3, as well as some key agents corresponding to their target. Other agents in early development that target EGFR and other pathways are listed in Table 1.

5.1 Irreversible next-generation EGFR TKIs

It has been suggested that simultaneous EGFR/HER2 inhibition may interrupt cooperative signaling between the two family members, which could lead to improved efficacy [84]. This has been demonstrated in preclinical studies with blockade of EGFR and HER2 in tumor cells [85, 86]. Early clinical data also suggest that inhibition of more than one member of the EGFR family versus targeting a single receptor leads to improved antitumor activity [84, 87]. Several agents that target multiple EGFR family members are in various phases of development; BIBW 2992 is currently the most advanced compound in this class.

BIBW 2992 is a small molecule irreversible inhibitor of EGFR/HER1 and HER2 [88]. In an ongoing phase II study (LUX-Lung 2) of NSCLC patients harboring *EGFR*-activating mutations, preliminary activity of BIBW 2992 was demonstrated in patients whose disease progressed following first-line chemotherapy and in patients whose disease reoccurred after neoadjuvant or adjuvant chemotherapy [89]. Preliminary results from that study showed a disease control rate (stable disease or partial or complete response) of 97% in 38 evaluable first-line patients [89]. Among 67 evaluable second-line patients, disease control rate was 97% [89]. Similar to other EGFR TKIs, diarrhea and skin adverse events were the most common adverse events [89, 90]. A phase IIb/III trial of BIBW 2992 plus best supportive care versus placebo plus best supportive care (LUX-Lung 1) is being conducted in patients with NSCLC who progressed after one to two lines of chemotherapy (including a platinum compound) and at least 12 weeks of either erlotinib or gefitinib treatment [91]. From May 2008 to April 2009, 482 patients have been screened and 367 patients have been randomized. This trial is ongoing, and as expected in patients treated with EGFR TKIs, diarrhea and skin adverse events are the most common drug-related adverse events that have been observed in a preliminary analysis. More recently, a phase III trial of BIBW 2992 as first-line therapy versus pemetrexed/cisplatin in patients with confirmed *EGFR*-activating mutations opened for enrollment in August 2009 (LUX-Lung 3) (www.clinicaltrials.gov identifier: NCT00949650).

PF00299804, an irreversible inhibitor of EGFR/HER1, HER2, and HER4, has shown preliminary antitumor activity and a predictable safety profile in an ongoing phase II study in patients with NSCLC after failure of prior chemotherapy and erlotinib [92]. In the phase II trial evaluating activity of PF00299804 in patients with advanced NSCLC who have progressed after one to two chemotherapy regimens and erlotinib, there were three confirmed partial responses and three patients with stable disease for >6 months [92]. Grade 3 toxicities included skin toxicity, diarrhea, fatigue, and vomiting [92]. A phase III trial of PF00299804 in patients with NSCLC who have progressed after receiving standard chemotherapy as well as erlotinib or gefitinib is planned. Several other phase II trials evaluating single-agent PF00299804 are also ongoing.

5.2 EGFR/VEGFR inhibitors

Several TKIs that target both EGFR and the VEGFR pathway are in development. It is postulated that simultaneous inhibition of multiple oncogenic pathways (proliferation, angiogenesis, etc.) will provide clinical benefit and reduce the risk of resistance. The most advanced of these compounds is vandetanib, an inhibitor of EGFR, VEGFR, and RET [93]. Three phase III studies have evaluated vandetanib in the treatment of NSCLC, but results have been mixed; two have evaluated vandetanib in combination with pemetrexed or docetaxel, and another trial compared vandetanib to erlotinib, all in patients with advanced NSCLC who had progressed after at least one chemotherapy regimen [93–95]. One of these trials (ZEAL, $N=534$) did not reach its primary endpoint of significantly improved PFS with the combination of vandetanib and pemetrexed versus pemetrexed alone [94]. Another (ZODIAC, $N=1,391$) demonstrated that vandetanib combined with docetaxel significantly improved PFS (HR, 0.79; 97.58% confidence interval, 0.70–0.90; $P<0.001$), the primary endpoint, but not overall survival, when compared with docetaxel alone [95]. The third phase III trial (ZEST, $N=1,240$), which compared vandetanib versus erlotinib, did not meet its primary endpoint of prolonged PFS with vandetanib; however, a preplanned non-inferiority analysis showed equivalent efficacy of vandetanib and erlotinib in that study [93]. Adverse events associated with vandetanib treatment include diarrhea, rash, neutropenia, and hypertension [93–95]. Another phase III study is currently evaluating vandetanib as monotherapy in patients with advanced NSCLC who have progressed after chemotherapy and erlotinib.

BMS-690514 is an inhibitor of EGFR/HER1, HER2, and VEGFR-1, VEGFR-2, and VEGFR-3. Results from a phase I/II trial ($N=60$) suggest activity of BMS-690514 in patients with NSCLC whose tumors have *EGFR* mutations [96]. Currently, a phase II trial of BMS-690514 versus erlotinib in previously treated patients with NSCLC is ongoing.

5.3 Inhibitors of targets downstream of EGFR

Mediators of downstream EGFR signaling are also being evaluated as potential targets for NSCLC therapy. Of these, the Ras-Raf-MEK-MAPK, PI3K-Akt-mTOR, and phospholipase C-PKC pathways have been most intensively studied (reviewed in [19]). One of the Ras-Raf-MEK-MAPK pathway inhibitors in development for the treatment of NSCLC is AZD6244, a MEK1/2 inhibitor [97]. Phase II trials are ongoing to evaluate AZD6244 in NSCLC patients with specific mutations such as B-raf, which has been shown in preclinical models to sensitize tumor cells to MEK inhibition [98]. Another phase II trial is testing AZD6244 versus pemetrexed in patients with NSCLC who have progressed after one or two lines of chemotherapy. PD-0325901, also an inhibitor of MEK [99], is in phase II trials for the treatment of advanced NSCLC.

PI3K-Akt-mTOR pathway inhibitors are being evaluated alone and in combination with EGFR-targeting agents for NSCLC. XL765, an inhibitor of PI3K and mTOR, is being

evaluated in combination with erlotinib in an ongoing phase Ib/II trial. Several mTOR inhibitors (e.g., temsirolimus or CCI-779, everolimus or RAD001) have shown initial activity in NSCLC [100]. CCI-779 and RAD001 are being studied alone and in combination with EGFR TKIs in phase II trials for NSCLC. AP23573, another mTOR inhibitor, is currently in phase II trials for treatment of NSCLC [101]. XL147 and GDC-0941 also inhibit PI3K and are being evaluated in phase I trials for treatment of solid tumors, including NSCLC. Several other PI3K inhibitors (e.g., PX866, SF1126, and BGT226) are also in development; however, many of these are not yet being evaluated specifically for lung cancer [102].

Through inhibition of PKC, the TKI enzastaurin abrogates the phospholipase C-PKC pathway. In a phase II trial ($N=55$) of enzastaurin for advanced NSCLC, the PFS endpoint was not achieved, but 35% of patients experienced disease stabilization [103]. Many phase II trials of enzastaurin in combination with cytotoxic agents for treatment of NSCLC are ongoing. ISIS 3521, an antisense agent targeting PKC [104], is currently in phase II and III trials for NSCLC in combination with cytotoxic agents.

While downstream mediators of EGFR provide many new opportunities for therapeutic intervention, next-generation EGFR inhibitors continue to be developed; currently, several anti-EGFR monoclonal antibodies are being evaluated for treatment of NSCLC (e.g., panitumumab, matuzumab).

6 Conclusions

Targeting the EGFR pathway has demonstrated clinical benefit for a select group of patients with NSCLC. However, the multitude of actual or potential targets for individualized therapy offers the prospect that most, if not all, patients will eventually benefit from carefully selected therapy targeted toward the EGFR pathway or another critical signaling pathway. Whether such therapy targets a single pathway or gene, a combination of targeted therapies, or a combination of targeted and conventional therapies remains to be determined. Such an individualized approach requires acquisition and testing of tumor tissue shortly before the selection of therapy. Tumor heterogeneity, genomic instability, and the development of resistance make it mandatory that tumor tissue be obtained for drug selection shortly before individualized therapy. Thus, other than for first-line therapy, a further biopsy or other form of intervention may be necessary. Individualized therapy also requires “reflex” testing of tissues—e.g., certain clinicopathological features trigger automatic or “reflex” testing. For instance, a diagnosis of adenocarcinoma of the lung combined with one or more other characteristics of *EGFR* mutant tumors (female gender, never smoking status, or East Asian ethnicity) would trigger “reflex” testing for mutations or other forms of deregulation of the EGFR pathway, with therapy decisions based on the results obtained. Such an approach is already practiced at certain leading medical centers including M.D. Anderson Cancer Center, Houston, TX, USA, and Memorial Sloan-Kettering Cancer Center, New York, NY, USA. When patients are informed that additional procedures are required to obtain tissue to guide personalized therapy, the vast majority are willing to participate in such trials. The advent of rapid, relatively cheap next-generation sequencing technologies for all or large parts of the genome will accelerate the identification of individualized targets.

Ideally, EGFR TKI therapy could be tailored to patients who are most likely to experience benefit, allowing other patients to receive different therapies appropriate for their tumor profile. Several potential molecular indicators have been identified, and optimization of assay technologies is in progress, but these are not yet widely used to direct treatment choices in NSCLC patients. As the transition to personalized medicine occurs, pathologists

will likely play a greater role in diagnostic decisions, and obtaining sufficient sample for testing upon initial biopsy will be critical. Moreover, it may be necessary to obtain subsequent biopsies when patients relapse or begin a new treatment regimen. In an effort to potentially overcome and prevent resistance to current EGFR-targeted agents, ongoing trials are evaluating new agents, such as EGFR/HER2 irreversible inhibitors and EGFR/VEGFR inhibitors. Hopefully, these new strategies for inhibiting multiple EGFR targets and/or multiple tumorigenic processes may eventually improve patient outcomes.

7 Key unanswered questions

The major unanswered questions are as follows:

What are the best targets for therapeutic interventions in the EGFR signaling pathway?

How do we identify the best targets in individual tumors? How do we get sufficient tissue for testing?

Can the tests be performed in a clinically relevant time frame at an acceptable cost?

What are the best drugs directed against these targets?

How do we combine targeted therapies with each other and with conventional therapies?

Are targeted therapies suitable for first-line therapies?

How do we overcome drug resistance?

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References

1. World Health Organization. Fact sheet no 310: the top ten causes of death. 2008 Nov.2008 http://www.who.int/mediacentre/factsheets/fs310_2008.pdf.
2. American Cancer Society. Cancer facts & figures, 2009. Atlanta, GA: American Cancer Society, Inc; 2009.
3. National Comprehensive Cancer Network. [Accessed 15 Dec 2009] NCCN Clinical Practice Guidelines in Oncology™: *Non-Small Cell Lung Cancer V.1.2010*. 2009. http://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf
4. Breathnach OS, Freidlin B, Conley B, Green MR, Johnson DH, Gandara DR, et al. Twenty-two years of phase III trials for patients with advanced non-small-cell lung cancer: sobering results. *Journal of Clinical Oncology*. 2001; 19(6):1734–1742. [PubMed: 11251004]
5. Carney DN. Lung cancer—time to move on from chemotherapy. *New England Journal of Medicine*. 2002; 346(2):126–128. [PubMed: 11784881]
6. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *Journal of Clinical Oncology*. 2004; 22(11):2184–2191. [PubMed: 15169807]

7. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *New England Journal of Medicine*. 2006; 355(24):2542–2550. [PubMed: 17167137]
8. Sibilio M, Kroismayr R, Lichtenberger BM, Natarajan A, Hecking M, Holcman M. The epidermal growth factor receptor: from development to tumorigenesis. *Differentiation*. 2007; 75(9):770–787. [PubMed: 17999740]
9. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000; 100(1):57–70. [PubMed: 10647931]
10. Hirsch FR, Varella-Garcia M, Cappuzzo F. Predictive value of EGFR and HER2 overexpression in advanced non-small-cell lung cancer. *Oncogene*. 2009; 28(Suppl 1):S32–S37. [PubMed: 19680294]
11. Hirsch FR, Scagliotti GV, Langer CJ, Varella-Garcia M, Franklin WA. Epidermal growth factor family of receptors in preneoplasia and lung cancer: perspectives for targeted therapies. *Lung Cancer*. 2003; 41(Suppl 1):S29–S42. [PubMed: 12867060]
12. Gazdar AF. Personalized medicine and inhibition of EGFR signaling in lung cancer. *New England Journal of Medicine*. 2009; 361(10):1018–1020. [PubMed: 19692681]
13. Grandal MV, Madhus IH. Epidermal growth factor receptor and cancer: control of oncogenic signalling by endocytosis. *Journal of Cellular and Molecular Medicine*. 2008; 12(5A):1527–1534. [PubMed: 18318691]
14. Herbst RS, Heymach JV, Lippman SM. Lung cancer. *New England Journal of Medicine*. 2008; 359(13):1367–1380. [PubMed: 18815398]
15. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. *New England Journal of Medicine*. 2008; 358(11):1160–1174. [PubMed: 18337605]
16. Laskin JJ, Sandler AB. Epidermal growth factor receptor: a promising target in solid tumours. *Cancer Treatment Reviews*. 2004; 30(1):1–17. [PubMed: 14766123]
17. Ho C, Laskin J. EGFR-directed therapies to treat non-small-cell lung cancer. *Expert Opinion on Investigational Drugs*. 2009; 18(8):1133–1145. [PubMed: 19572809]
18. Solomon B, Varella-Garcia M, Camidge DR. ALK gene rearrangements: a new therapeutic target in a molecularly defined subset of non-small cell lung cancer. *Journal of Thoracic Oncology*. 2009; 4(12):1450–1454. [PubMed: 20009909]
19. Milano A, De Iaffaioli RV, Caponigro F. Downstream intracellular effectors of epidermal growth factor receptor as targets for anticancer therapy. *Expert Opinion on Therapeutic Targets*. 2007; 11(6):771–782. [PubMed: 17504015]
20. Quesnelle KM, Boehm AL, Grandis JR. STAT-mediated EGFR signaling in cancer. *Journal of Cellular Biochemistry*. 2007; 102(2):311–319. [PubMed: 17661350]
21. Silva CM. Role of STATs as downstream signal transducers in Src family kinase-mediated tumorigenesis. *Oncogene*. 2004; 23(48):8017–8023. [PubMed: 15489919]
22. Modi S, Seidman AD. An update on epidermal growth factor receptor inhibitors. *Current Oncology Reports*. 2002; 4(1):47–55. [PubMed: 11734113]
23. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, Bremmes RM, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *Journal of Clinical Oncology*. 2003; 21(20):3798–3807. [PubMed: 12953099]
24. Ohsaki Y, Tanno S, Fujita Y, Toyoshima E, Fujiuchi S, Nishigaki Y, et al. Epidermal growth factor receptor expression correlates with poor prognosis in non-small cell lung cancer patients with p53 overexpression. *Oncology Reports*. 2000; 7(3):603–607. [PubMed: 10767376]
25. John T, Liu G, Tsao MS. Overview of molecular testing in non-small-cell lung cancer: mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors. *Oncogene*. 2009; 28(Suppl 1):S14–S23. [PubMed: 19680292]
26. Eberhard DA, Giaccone G, Johnson BE. Biomarkers of response to epidermal growth factor receptor inhibitors in Non-Small-Cell Lung Cancer Working Group: standardization for use in the clinical trial setting. *Journal of Clinical Oncology*. 2008; 26(6):983–994. [PubMed: 18281673]

27. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Franklin WA, Dziadziuszko R, Thatcher N, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. *Journal of Clinical Oncology*. 2006; 24(31):5034–5042. [PubMed: 17075123]
28. Parra HS, Cavina R, Latteri F, Zucali PA, Campagnoli E, Morenghi E, et al. Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ('Iressa', ZD1839) in non-small-cell lung cancer. *British Journal of Cancer*. 2004; 91(2):208–212. [PubMed: 15187994]
29. Perez-Soler R, Chachoua A, Hammond LA, Rowinsky EK, Huberman M, Karp D, et al. Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. *Journal of Clinical Oncology*. 2004; 22(16):3238–3247. [PubMed: 15310767]
30. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, et al. Erlotinib in lung cancer—molecular and clinical predictors of outcome. *New England Journal of Medicine*. 2005; 353(2):133–144. [PubMed: 16014883]
31. Rego RL, Foster NR, Smyrk TC, Le M, O'Connell MJ, Sargent DJ, et al. Prognostic effect of activated EGFR expression in human colon carcinomas: comparison with EGFR status. *British Journal of Cancer*. 2010; 102:165–172. [PubMed: 19997103]
32. Isola J, Tanner M, Forsyth A, Cooke TG, Watters AD, Bartlett JM. Interlaboratory comparison of HER-2 oncogene amplification as detected by chromogenic and fluorescence *in situ* hybridization. *Clinical Cancer Research*. 2004; 10(14):4793–4798. [PubMed: 15269154]
33. Zhu CQ, da Cunha SG, Ding K, Sakurada A, Cutz JC, Liu N, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. *Journal of Clinical Oncology*. 2008; 26(26):4268–4275. [PubMed: 18626007]
34. Hirsch FR, Herbst RS, Olsen C, Chansky K, Crowley J, Kelly K, et al. Increased EGFR gene copy number detected by fluorescent *in situ* hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy. *Journal of Clinical Oncology*. 2008; 26(20):3351–3357. [PubMed: 18612151]
35. Hirsch FR, Varella-Garcia M, Cappuzzo F, McCoy J, Bemis L, Xavier AC, et al. Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib. *Annals of Oncology*. 2007; 18(4):752–760. [PubMed: 17317677]
36. Dziadziuszko R, Witta SE, Cappuzzo F, Park S, Tanaka K, Danenberg PV, et al. Epidermal growth factor receptor messenger RNA expression, gene dosage, and gefitinib sensitivity in non-small cell lung cancer. *Clinical Cancer Research*. 2006; 12(10):3078–3084. [PubMed: 16707605]
37. Gandhi J, Zhang J, Xie Y, Soh J, Shigematsu H, Zhang W, et al. Alterations in genes of the EGFR signaling pathway and their relationship to EGFR tyrosine kinase inhibitor sensitivity in lung cancer cell lines. *PLoS ONE*. 2009; 4(2):e4576. [PubMed: 19238210]
38. Bell DW, Lynch TJ, Haserlat SM, Harris PL, Okimoto RA, Brannigan BW, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *Journal of Clinical Oncology*. 2005; 23(31):8081–8092. [PubMed: 16204011]
39. Dziadziuszko R, Holm B, Skov BG, Osterlind K, Sellers MV, Franklin WA, et al. Epidermal growth factor receptor gene copy number and protein level are not associated with outcome of non-small-cell lung cancer patients treated with chemotherapy. *Annals of Oncology*. 2007; 18(3):447–452. [PubMed: 17082511]
40. Takano T, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U, et al. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *Journal of Clinical Oncology*. 2005; 23(28):6829–6837. [PubMed: 15998907]
41. Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. *Oncogene*. 2009; 28(Suppl 1):S24–S31. [PubMed: 19680293]
42. Costa DB, Kobayashi S, Tenen DG, Huberman MS. Pooled analysis of the prospective trials of gefitinib monotherapy for EGFR-mutant non-small cell lung cancers. *Lung Cancer*. 2007; 58(1):95–103. [PubMed: 17610986]

43. Inoue A, Suzuki T, Fukuhara T, Maemondo M, Kimura Y, Morikawa N, et al. Prospective phase II study of gefitinib for chemotherapy-naïve patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *Journal of Clinical Oncology*. 2006; 24(21): 3340–3346. [PubMed: 16785471]
44. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *New England Journal of Medicine*. 2004; 350(21):2129–2139. [PubMed: 15118073]
45. Miller VA, Riely GJ, Zakowski MF, Li AR, Patel JD, Heelan RT, et al. Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib. *Journal of Clinical Oncology*. 2008; 26(9):1472–1478. [PubMed: 18349398]
46. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004; 304(5676):1497–1500. [PubMed: 15118125]
47. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(36):13306–13311. [PubMed: 15329413]
48. Sequist LV, Martins RG, Spigel D, Grunberg SM, Spira A, Janne PA, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. *Journal of Clinical Oncology*. 2008; 26(15):2442–2449. [PubMed: 18458038]
49. Tamura K, Okamoto I, Kashii T, Negoro S, Hirashima T, Kudoh S, et al. Multicentre prospective phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403). *British Journal of Cancer*. 2008; 98(5):907–914. [PubMed: 18283321]
50. Taron M, Ichinose Y, Rosell R, Mok T, Massuti B, Zamora L, et al. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. *Clinical Cancer Research*. 2005; 11(16):5878–5885. [PubMed: 16115929]
51. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *New England Journal of Medicine*. 2009; 361(10):947–957. [PubMed: 19692680]
52. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *New England Journal of Medicine*. 2009; 361(10):958–967. [PubMed: 19692684]
53. Greulich H, Chen TH, Feng W, Janne PA, Alvarez JV, Zappaterra M, et al. Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. *PLoS Medicine*. 2005; 2(11):e313. [PubMed: 16187797]
54. Molina-Vila MA, Bertran-Alamillo J, Reguart N, Taron M, Castella E, Llatjos M, et al. A sensitive method for detecting EGFR mutations in non-small cell lung cancer samples with few tumor cells. *Journal of Thoracic Oncology*. 2008; 3(11):1224–1235. [PubMed: 18978556]
55. DxS Diagnostic Innovations. [Accessed 28 Dec 2009] DxS EGFR mutation test kit: for the detection of 29 mutations in the epidermal growth factor receptor (EGFR) gene. Instructions for use. 2009. Product codes: EG-03 and EG-04. Instructions Version: RU001b. Date of Revision: July 2009.
<http://www.dxsdiagnostics.com/Site/PDF/CMP/RUO-EGFR29/IFU-EGFR29-RUO-US.pdf>
56. Mack PC, Holland WS, Burich RA, Sangha R, Solis LJ, Li Y, et al. EGFR mutations detected in plasma are associated with patient outcomes in erlotinib plus docetaxel-treated non-small cell lung cancer. *Journal of Thoracic Oncology*. 2009; 4(12):1466–1472. [PubMed: 19884861]
57. Zhang X, Chang A. Molecular predictors of EGFR-TKI sensitivity in advanced non-small cell lung cancer. *International Journal of Medical Sciences*. 2008; 5(4):209–217. [PubMed: 18645621]
58. Ladanyi M, Pao W. Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. *Modern Pathology*. 2008; 21(Suppl 2):S16–S22. [PubMed: 18437168]

59. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nature Reviews Cancer*. 2005; 5(5):341–354.
60. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *Journal of Clinical Oncology*. 2003; 21(14):2787–2799. [PubMed: 12860957]
61. Kelly K, Chansky K, Gaspar LE, Albain KS, Jett J, Ung YC, et al. Phase III trial of maintenance gefitinib or placebo after concurrent chemoradiotherapy and docetaxel consolidation in inoperable stage III non-small-cell lung cancer: SWOG S0023. *Journal of Clinical Oncology*. 2008; 26(15): 2450–2456. [PubMed: 18378568]
62. Gatzemeier U, Pluzanska A, Szczesna A, Kaukel E, Roubec J, De Rosa F, et al. Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva Lung Cancer Investigation Trial. *Journal of Clinical Oncology*. 2007; 25(12):1545–1552. [PubMed: 17442998]
63. Pirker R, Pereira JR, Szczesna A, von Pawel J, Krzakowski M, Ramlau R, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet*. 2009; 373(9674):1525–1531. [PubMed: 19410716]
64. Merck, KGaA. [Accessed 15 Dec 2009] News release. November 19, 2009. CHMP opinion for erbitux in advanced non-small cell lung cancer. 2009. [http://news.merck.de/N/0/95F233DCB18F57F1C1257673003661FC/\\$File/CHMP_neg_En.pdf](http://news.merck.de/N/0/95F233DCB18F57F1C1257673003661FC/$File/CHMP_neg_En.pdf)
65. Thatcher N, Chang A, Parikh P, Rodrigues PJ, Ciuleanu T, von Pawel J, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet*. 2005; 366(9496):1527–1537. [PubMed: 16257339]
66. Shepherd FA, Rodrigues PJ, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *New England Journal of Medicine*. 2005; 353(2): 123–132. [PubMed: 16014882]
67. Tarceva® (erlotinib) tablets, oral [package insert]. Melville, NY: OSI Pharmaceuticals Inc; 2009.
68. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *New England Journal of Medicine*. 2005; 352(8):786–792. [PubMed: 15728811]
69. Kosaka T, Yatabe Y, Endoh H, Yoshida K, Hida T, Tsuboi M, et al. Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. *Clinical Cancer Research*. 2006; 12(19):5764–5769. [PubMed: 17020982]
70. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Medicine*. 2005; 2(3):e73. [PubMed: 15737014]
71. Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *Journal of Clinical Oncology*. 2005; 23(25):5900–5909. [PubMed: 16043828]
72. Pao W, Wang TY, Riely GJ, Miller VA, Pan Q, Ladanyi M, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Medicine*. 2005; 2(1):e17. [PubMed: 15696205]
73. Balak MN, Gong Y, Riely GJ, Somwar R, Li AR, Zakowski MF, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. *Clinical Cancer Research*. 2006; 12(21):6494–6501. [PubMed: 17085664]
74. Suda K, Onozato R, Yatabe Y, Mitsudomi T. EGFR T790M mutation: a double role in lung cancer cell survival? *Journal of Thoracic Oncology*. 2009; 4(1):1–4. [PubMed: 19096299]
75. Maheswaran S, Sequist LV, Nagrath S, Ullus L, Brannigan B, Collura CV, et al. Detection of mutations in EGFR in circulating lung-cancer cells. *New England Journal of Medicine*. 2008; 359(4):366–377. [PubMed: 18596266]
76. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or

- erlotinib. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(52):20932–20937. [PubMed: 18093943]
77. Aita M, Fasola G, Defferrari C, Brianti A, Bello MG, Follador A, et al. Targeting the VEGF pathway: antiangiogenic strategies in the treatment of non-small cell lung cancer. *Critical Reviews in Oncology/Hematology*. 2008; 68(3):183–196. [PubMed: 18606548]
 78. Alvarez RH, Kantarjian HM, Cortes JE. Biology of platelet-derived growth factor and its involvement in disease. *Mayo Clinic Proceedings*. 2006; 81(9):1241–1257. [PubMed: 16970222]
 79. Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. *Journal of Clinical Investigation*. 2008; 118(7):2609–2619. [PubMed: 18568074]
 80. Kono SA, Marshall ME, Ware KE, Heasley LE. The fibroblast growth factor receptor signaling pathway as a mediator of intrinsic resistance to EGFR-specific tyrosine kinase inhibitors in non-small cell lung cancer. *Drug Resistance Updates*. 2009; 12(4–5):95–102. [PubMed: 19501013]
 81. Kwak EL, Sordella R, Bell DW, Godin-Heymann N, Okimoto RA, Brannigan BW, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(21):7665–7670. [PubMed: 15897464]
 82. Burris HA III. Shortcomings of current therapies for non-small-cell lung cancer: unmet medical needs. *Oncogene*. 2009; 28(Suppl 1):S4–S13. [PubMed: 19680296]
 83. Britten CD. Targeting ErbB receptor signaling: a pan-ErbB approach to cancer. *Molecular Cancer Therapeutics*. 2004; 3(10):1335–1342. [PubMed: 15486201]
 84. Yang C-H, Shih J-Y, Su W-C, Hsia T-C, Ho C-L, Dudek AZ, et al. BIBW 2992, a novel irreversible EGFR/HER2 tyrosine kinase inhibitor, in chemonaïve patients with adenocarcinoma of the lung and activating EGFR mutations (LUX-Lung 2). *Journal of Thoracic Oncology*. 2009; 4(9 suppl 1):S294–S295. Abstract A3.3.
 85. Xu H, Yu Y, Marciniak D, Rishi AK, Sarkar FH, Kucuk O, et al. Epidermal growth factor receptor (EGFR)-related protein inhibits multiple members of the EGFR family in colon and breast cancer cells. *Molecular Cancer Therapeutics*. 2005; 4(3):435–442. [PubMed: 15767552]
 86. Ye D, Mendelsohn J, Fan Z. Augmentation of a humanized anti-HER2 mAb 4D5 induced growth inhibition by a human-mouse chimeric anti-EGF receptor mAb C225. *Oncogene*. 1999; 18(3):731–738. [PubMed: 9989823]
 87. Shih J-Y, Yang C-H, Su W-C, Hsia T-C, Tsia C-M, Chen Y-M, et al. A phase II study of BIBW 2992, a novel irreversible dual EGFR and HER2 TKI, in patients with adenocarcinoma of the lung and activating EGFR mutations after failure of one line of chemotherapy (LUX-Lung 2). *Journal of Clinical Oncology*. 2009; 27(suppl):15S, Abstract 8013.
 88. Eskens FA, Mom CH, Planting AS, Gietema JA, Amelsberg A, Huisman H, et al. A phase I dose escalation study of BIBW 2992, an irreversible dual inhibitor of epidermal growth factor receptor 1 (EGFR) and 2 (HER2) tyrosine kinase in a 2-week on, 2-week off schedule in patients with advanced solid tumours. *British Journal of Cancer*. 2008; 98(1):80–85. [PubMed: 18026190]
 89. Yang, C-H.; Shih, J-Y.; Su, W-C.; Hsia, T-C.; Ho, C-L.; Dudek, AZ., et al. BIBW 2992, a novel irreversible EGFR/HER2 tyrosine kinase inhibitor, in chemonaïve patients with adenocarcinoma of the lung and activating EGFR mutations (LUX-Lung 2). Oral presentation at: 13th World Conference on Lung Cancer of the International Association for the Study of Lung Cancer; July 31–August 4, 2009; San Francisco, CA.
 90. Shih, J-Y.; Yang, C-H.; Su, W-C.; Hsia, T-C.; Tsai, C-M.; Chen, Y-M., et al. A phase II study of BIBW 2992, a novel irreversible dual EGFR and HER2 TKI, in patients with adenocarcinoma of the lung and activating EGFR mutations after failure of one line of chemotherapy (LUX-Lung 2). Poster presented at: 45th Annual Meeting of the American Society of Clinical Oncology; May 29–June 2, 2009; Orlando, FL.
 91. Yang, C-H.; Hirsh, V.; Cadranel, J.; Chen, Y-M.; Park, K.; Kim, S-W., et al. Phase IIb/III double-blind randomized trial of BIBW 2992, an irreversible, dual inhibitor of EGFR (HER1) and HER2 + BSC versus placebo + BSC in patients with non-small cell lung cancer failing 1–2 lines of chemotherapy and erlotinib or gefitinib (LUX-Lung 1): a preliminary report. Poster presented at: 45th Annual Meeting of the American Society of Clinical Oncology; May 29–June 2, 2009; Orlando, FL.

92. Janne PA, Reckamp K, Koczywas M, Camidge DR, Engelman JA, Khuri F, et al. A phase 2 trial of PF-00299804 (PF299), an oral irreversible HER tyrosine kinase inhibitor (TKI), in patients (pts) with advanced NSCLC after failure of prior chemotherapy and erlotinib: preliminary efficacy and safety results. *Journal of Thoracic Oncology*. 2009; 4(9 suppl 1):S293–S294. Abstract A3.1.
93. Natale RB, Thongprasert S, Greco FA, et al. Vandetanib versus erlotinib in patients with advanced non-small cell lung cancer (NSCLC) after failure of at least one prior cytotoxic chemotherapy: a randomized, double-blind phase III trial (ZEST). *Journal of Clinical Oncology*. 2009; 27(suppl):Abstract 8009.
94. De Boer R, Arrieta O, Gottfried M, et al. Vandetanib plus pemetrexed versus pemetrexed as second-line therapy in patients with advanced non-small cell lung cancer (NSCLC): a randomized, double-blind phase III trial (ZEAL). *Journal of Clinical Oncology*. 2009; 27(suppl):409s, Abstract 8010.
95. Herbst RS, Sun Y, Korfee S, et al. Vandetanib plus docetaxel versus docetaxel as second-line treatment for patients with advanced non-small cell lung cancer (NSCLC): a randomized, double-blind phase III trial (ZODIAC). *Journal of Clinical Oncology*. 2009; 27(suppl):807s, Abstract CRA8003.
96. Bahleda R, Soria JC, Harbison CT, et al. Tumor regression and pharmacodynamic (PD) biomarker validation in non-small cell lung cancer (NSCLC) patients treated with the ErbB/VEGFR inhibitor BMS-690514. *Journal of Clinical Oncology*. 2009; 27(suppl):431s, Abstract 8098.
97. Shannon AM, Telfer BA, Smith PD, Babur M, Logie A, Wilkinson RW, et al. The mitogen-activated protein/extracellular signal-regulated kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) enhances the radiation responsiveness of lung and colorectal tumor xenografts. *Clinical Cancer Research*. 2009; 15(21):6619–6629. [PubMed: 19843666]
98. Friday BB, Yu C, Dy GK, Smith PD, Wang L, Thibodeau SN, et al. BRAF V600E disrupts AZD6244-induced abrogation of negative feedback pathways between extracellular signal-regulated kinase and Raf proteins. *Cancer Research*. 2008; 68(15):6145–6153. [PubMed: 18676837]
99. Barrett SD, Bridges AJ, Dudley DT, Saltiel AR, Fergus JH, Flamme CM, et al. The discovery of the benzhydroxamate MEK inhibitors CI-1040 and PD 0325901. *Bioorganic & Medicinal Chemistry Letters*. 2008; 18(24):6501–6504. [PubMed: 18952427]
100. Gridelli C, Maione P, Rossi A. The potential role of mTOR inhibitors in non-small cell lung cancer. *The Oncologist*. 2008; 13(2):139–147. [PubMed: 18305058]
101. Mita MM, Mita AC, Chu QS, Rowinsky EK, Fetterly GJ, Goldston M, et al. Phase I trial of the novel mammalian target of rapamycin inhibitor deforolimus (AP23573; MK-8669) administered intravenously daily for 5 days every 2 weeks to patients with advanced malignancies. *Journal of Clinical Oncology*. 2008; 26(3):361–367. [PubMed: 18202410]
102. Wu P, Liu T, Hu Y. PI3K inhibitors for cancer therapy: what has been achieved so far? *Current Medicinal Chemistry*. 2009; 16(8):916–930. [PubMed: 19275602]
103. Oh Y, Herbst RS, Burris H, Cleverly A, Musib L, Lahn M, et al. Enzastaurin, an oral serine/threonine kinase inhibitor, as second- or third-line therapy of non-small-cell lung cancer. *Journal of Clinical Oncology*. 2008; 26(7):1135–1141. [PubMed: 18309949]
104. Villalona-Calero MA, Ritch P, Figueroa JA, Otterson GA, Belt R, Dow E, et al. A phase I/II study of LY900003, an antisense inhibitor of protein kinase C- α , in combination with cisplatin and gemcitabine in patients with advanced non-small cell lung cancer. *Clinical Cancer Research*. 2004; 10(18 Pt 1):6086–6093. [PubMed: 15447994]
105. Baselga J, Rojo F, Dumez H. Phase I study of AEE788, a novel multitargeted inhibitor of ErbB and VEGF receptor family tyrosine kinases: a pharmacokinetic (PK)-pharmacodynamic (PD) study to identify the optimal therapeutic dose regimen. *Journal of Clinical Oncology*. 2005; 23:Abstract 3028.
106. Rizvi NA, Kris MG, Miller VA. Activity of XL647 in clinically selected NSCLC patients (pts) enriched for the presence of EGFR mutations: results from phase 2. *Journal of Clinical Oncology*. 2008; 26:Abstract 8053.
107. Yoshimura N, Kudoh S, Kimura T, Mitsuoka S, Matsuura K, Hirata K, et al. EKB-569, a new irreversible epidermal growth factor receptor tyrosine kinase inhibitor, with clinical activity in

- patients with non-small cell lung cancer with acquired resistance to gefitinib. *Lung Cancer*. 2006; 51(3):363–368. [PubMed: 16364494]
108. Suzuki T, Fujii A, Ohya J, Amano Y, Kitano Y, Abe D, et al. Pharmacological characterization of MP-412 (AV-412), a dual epidermal growth factor receptor and ErbB2 tyrosine kinase inhibitor. *Cancer Science*. 2007; 98(12):1977–1984. [PubMed: 17888033]
109. Gratacap MP, Martin V, Valera MC, Allart S, Garcia C, Sie P, et al. The new tyrosine-kinase inhibitor and anti-cancer drug dasatinib reversibly affects platelet activation *in vitro* and *in vivo*. *Blood*. 2009; 114(9):1884–1892. [PubMed: 19494352]
110. Natale RB, Bodkin D, Govindan R, Sleckman BG, Rizvi NA, Capo A, et al. Vandetanib versus gefitinib in patients with advanced non-small-cell lung cancer: results from a two-part, double-blind, randomized phase II study. *Journal of Clinical Oncology*. 2009; 27(15):2523–2529. [PubMed: 19332730]
111. Pirker R, Szczesna A, von Pawel J, et al. FLEX: a randomized, multicenter, phase III study of cetuximab in combination with cisplatin/vinorelbine (CV) versus CV alone in the first-line treatment of patients with advanced non-small cell lung cancer (NSCLC). *Proceedings of the American Society of Clinical Oncology*. 2008; 26:Abstract 3.

Fig. 1. Cellular effects resulting from activation of the EGFR pathway. EGFR signaling mediates the activation of a variety of cellular processes associated with carcinogenesis. All of the hallmarks of cancer are activated with the exception of limitless replicative potential

Fig. 2.

Mechanisms of EGFR resistance to TKIs in NSCLCs. (a) illustrates TKI-sensitive EGFR, where inhibition results in abrogation of EGFR signaling. TKIs may be ineffective for inhibiting EGFR signaling if specific *EGFR* mutations, such as T790M (b), amplification of *MET* (c), or *KRAS* mutation (d) occurs. I would like to thank Dr. Lecia Sequist, MD, MPH, and Dr. Jeffrey Settleman, PhD, for suggestion of the concept for Fig. 2 illustration

Fig. 3.

Potential targets for inhibition within the EGFR signaling pathway. Multiple points along the EGFR signaling pathway, indicated with *arrows*, are currently being evaluated as therapeutic targets in the treatment of NSCLC. Inhibition of EGFR activation can occur by targeting EGFR itself and/or its downstream mediators. Small molecule TKIs and other agents are shown associated with their respective targets. Figure adapted with permission from [12]

Table 1

EGFR pathway inhibitors for treatment of NSCLC [61, 62, 89, 92, 96, 97, 99–101, 103–111]

Agent	Binding	Target(s)	Phase
BIBW 2992	Irreversible	EGFR, HER2	III
PF00299804	Irreversible	EGFR, HER2, HER4	III
EKB-569 ^a	Irreversible	EGFR/HER2/HER4 TKI	II
Erlotinib	Reversible	EGFR	Approved
Gefitinib	Reversible	EGFR	See footnote ^b
Cetuximab	Reversible	EGFR	III
Vandetanib	Reversible	EGFR, VEGFR, RET	III
Enzastaurin	Reversible	PKC	II/III
ISIS 3521	Reversible	PKC	II/III
BMS-690514	Reversible	EGFR, HER2, VEGFR-1, VEGFR-2 and VEGFR-3	II
AZD6244	Reversible	MEK	II
PD-0325901	Reversible	MEK	II
Matuzumab	Reversible	EGFR	II
AP23573	Reversible	mTOR	II
XL765	Reversible	PI3K, mTOR	I/II
XL647 ^a	Reversible	EGFR/HER2/VEGFR	II
Dasatinib	Reversible	Src, PDGFR, c-kit	II
Panitumumab	Reversible	EGFR	I/II
Temsirolimus	Reversible	mTOR	I/II
Everolimus	Reversible	mTOR	I/II
AEE788	Reversible	EGFR/VEGFR	I/II
AV-412/MP-412	Reversible	EGFR/HER2	I
XL147	Reversible	PI3K	I
GDC-0941	Reversible	PI3K	I

PDGFR platelet-derived growth factor receptor, *c-kit* stem cell factor receptor^aThese compounds may not continue clinical development for NSCLC therapy^bGefitinib is currently only available for use in the USA in patients who are or have previously benefited from gefitinib treatment